



Genome Editing and the Future of Farming

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Opportunities to improve health, production efficiency and sustainability through applied gene editing

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ABSTRACT

Recent advances in gene editing technologies and in the application of these technologies to livestock animals have created a wealth of opportunity for improving animal health and well-being and thereby the sustainability of animal protein production. I review two technology examples in porcine and bovine systems that Genus plc. is engaged in advancing. In pigs, recent published work has demonstrated that a simple edit producing a loss of function variant for the gene product CD163 can produce full resistance to the devastating disease porcine reproductive and respiratory syndrome virus (PRRSv)¹. In cattle, a more subtle edit, involving an edit of the -5 amino acid before the signal cleavage site of the CD18 gene product from glutamine to glycine has been shown in cell model systems to confer resistance to the *Mannheimia haemolytica* leukotoxin², and hypothesised to improve resilience to bovine respiratory disease (BRD). Among other challenges, the development and successful commercialization of these types of gene editing technologies will require the creation of multiple, consistent, reproducible edits in commercial founder lines of elite genetics. The practical challenges of deploying these technologies in beef, dairy and pork production systems are also considered.

INTRODUCTION

As noted by other authors the relatively recent emergence of efficient gene editing reagents has created a resurgence in interest in livestock genome engineering³. Pigs⁴, cattle³ and sheep³ have all been successfully gene edited and the range of genetic changes has progressed from loss of function variants⁵ to allele introgression within⁶ and across⁷ species. Applications are rapidly advancing in the domain of improving animal health and well-being with published proof of concept results addressing high impact diseases like porcine reproductive and respiratory syndrome virus (PRRSv) in swine¹, and day to day management challenges such as dehorning of dairy cattle⁶. In addition to these results there are a number of research efforts ongoing to address multiple livestock health challenges.

One of the most promising examples of the use of gene editing to positively impact livestock health is the recent demonstration that gene edited pigs lacking the CD163 gene product are protected from infection by the PRRS virus¹. The disease (now called porcine reproductive and respiratory syndrome) was first recognised in the late 1980s and is characterised by rapid onset abortions and fertility loss as well as high mortality in young pigs and loss of productivity due to respiratory infections⁸. Early in the 1990s a novel virus was isolated from infected sows and pigs in the Netherlands by Dutch scientists termed the Lelystad virus⁹ and subsequently shown definitely to be the causal agent¹⁰. Despite the isolation of and characterization of the virus, the disease has persisted and affects pork production in most parts of the globe¹¹. The role of the porcine CD163 gene product in PRRSv infection has been reviewed by Welch and Calvert¹² in 2010. Whitworth *et al.*¹ demonstrate convincingly that the CD163 gene product is required for infection in pigs and that pigs in which the gene product was missing, through gene editing with CRISPR/Cas9¹³, behaved as fully resistant and displayed no observable phenotypic abnormalities¹.

Likewise in cattle, opportunities exist to improve animal health or resilience to disease through genome editing. Bovine respiratory disease (BRD) is a complex, multifactorial disease involving multiple viral and bacterial factors¹⁴. *M. haemolytica* is one of the main bacterial organisms isolated from cattle with BRD and is thought to be the major bacterial factor in BRD. A ruminant

specific leukotoxin has been identified as a principal component in the impact of the disease¹⁵. The uncleaved signal peptide of the bovine CD18 gene has been shown in cell system assays to be required for haemolysis to occur, and restoration of cleavage through engineering the introduction of a Q>G mutation can prevent haemolysis². These authors hypothesise that introducing this change into cattle and other ruminants could improve the resilience of livestock to BRD². This is a hypothesis we are pursuing at Genus plc.

Clearly both these examples require further research and development to fully realise their potential in livestock, but in addition to the challenges of advancing these types of technologies at the molecular cellular and organismal levels, several additional systemic challenges have to be overcome for the successful commercialization of these types of technologies in modern livestock production systems.

DISCUSSION

Systemic challenges to commercially successful gene editing in livestock.

In addition to the large challenges of simply discovering technologies, like those introduced above, which can benefit livestock, producers and society, there are challenges inherent to livestock production itself and to the application of genetic technologies in agriculture that deserve consideration. Three of these challenges I will consider here are: technology regulation, technology acceptance and the production system expectations.

TECHNOLOGY REGULATION

Both the above cited examples can be produced with technologies that are collectively referred to today as “gene editing” and both can be realised at the commercial level in livestock without the introduction of DNA sequences from other species. This distinguishes these types of genetic changes from the more widely available “transgenic” or “GM” technologies that are currently commercially ubiquitous in much of row crop agriculture in the Americas and are equally technically feasible¹⁶, though far less commercially impactful in livestock. Gene editing, however, used in this context is quite new and the regulatory paradigm is evolving. Delays in

clarity on the process by which regulation will occur can be more problematic and costly for technology development than the regulation process itself. In the event that gene editing is regulated under the currently established paradigms for transgenic technologies, regulatory costs and regulatory processes will present unique challenges to the application in livestock. As of November 2016, the United States Food and Drug Administration (FDA) has continued to state that it will regulate gene edited livestock under new animal drug provisions of the Federal Food Drug & Cosmetic Act^{17,18,19}. The costs of regulation and regulatory approvals for GM technologies are well studied and are now sufficiently mature to provide a useful expectation of what a modern regulatory dossier and approval process will likely entail. A consultancy study published in 2011 by Phillips McDougal for CropLife International ("Cost Of Bringing A Biotech Crop To Market"²⁰) puts the cost of regulatory science and regulatory engagement for a single GM crop product at \$35 million USD. This cost clearly sets a high bar for necessary economic value of a trait that is to be taken through the regulatory process and brought to market. Costs of this scale are likely to be one reason why only very high value technologies are brought to market, and why they are brought so frequently, by enlarge, by multinational businesses with the economic resources necessary to bear these costs. Less costly alternatives are being discussed in some jurisdictions and may positively impact the distribution of the benefits of gene editing technologies.

TECHNOLOGY ACCEPTANCE

Public acceptance, though potentially higher than for transgenics, remains a largely unexplored question. Attitudes and interest in technology in food production, and in animal protein production specifically, vary greatly. Regardless of their positions on specific practices many who work with livestock recognise that, with some variation by species and production system, people relate to livestock and livestock relate to people and it therefore is natural that concerns about technology use in animals is somewhat different than concerns about technology use in agricultural plants. Genus is focusing its gene editing development efforts on targets with tangible benefits for livestock health and well-being, because in addition to the evident benefit for farmers and producers, we expect that these technologies, with tangible animal benefit,

may have higher acceptance among members of the public who take active interest in the production of their food and the well-being of animals involved in that production.

THE CHALLENGES OF MODERN PRODUCER EXPECTATIONS

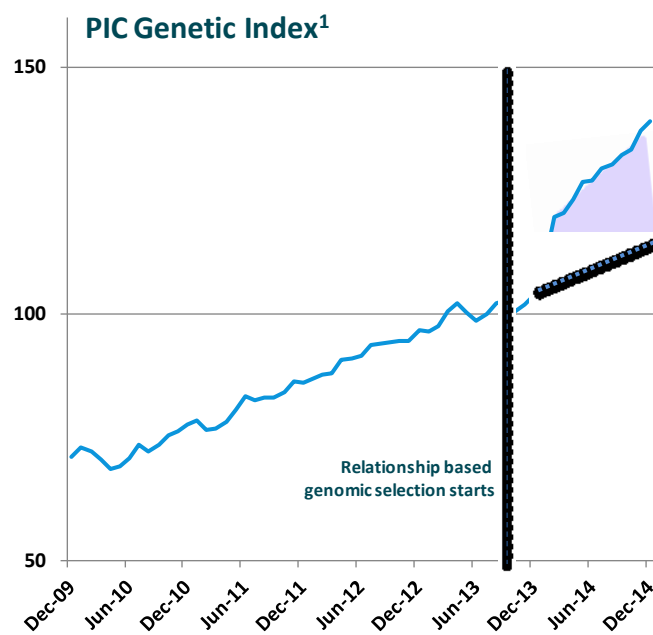
Genetic improvement in livestock species has been going on for 100s of years but improvement towards specific economic outcomes with modern statistical tools originated in the mid-20th Century²¹. Most recently, the application of genomic selection has further accelerated genetic gain and produced in many livestock systems a farmer expectation of continuous genetic improvement that requires resource and focus to deliver. The impacts of genomic selection on the rate of genetic improvement in Holstein dairy cattle were recently reviewed by García-Ruiz *et al.*²². They conclude that rates of genetic gain have improved dramatically across all traits with the largest impacts in lowly heritable traits¹⁸. One common selection index for US dairy cattle profitability is the Net Merit Index (NM\$)²³ which has units in terms of US dollars. Since the implementation of genomic selection NM\$ of the average Holstein sire in stud in the US has increased markedly and at a rate exceeding 50NM\$ per year. In the PIC subsidiary of Genus plc., which has been improving porcine genetics for more than 40 years and implemented relationship based genomic selection across its lines in 2013 (Figure 1), the PIC index is a proprietary breeding index based on total economic value in pork production. Rates of genetic improvement seen in our business since the implementation of genomic selection meet or exceed those seen in the dairy system. The challenge presented for the successful introduction of a gene edit in both these genetic systems stems from the simple challenge of creating the edits in the most competitive germplasm available and disseminating those genetics before they are obsolete. If, for example, we assume conservatively that PRRSv costs pork production on average 10% productivity per year, and we assume that PIC[®] genetics are improving at a rate of 5% per year, only two years of genetic lag would make introduction of the technology a break even proposition at the producer level, on average. This challenge is further increased by the fact that PIC improves nine distinct lines which are combined through production pyramids to produce crossbred sows, and crossbred boars, which are then further bred to produce terminal pigs for meat production (Figure 2). Because of the recessive nature of the resistance

phenotype, multiple edited pigs from the most elite genetics from multiple lines (in this example four) must be produced to create the possibility of having terminal pigs that are homozygous for the CD163 edit. Clearly, the nature of regulation and the efficiency of editing processes will be important components in the ultimate overall success of delivery of these technologies.

CONCLUSIONS

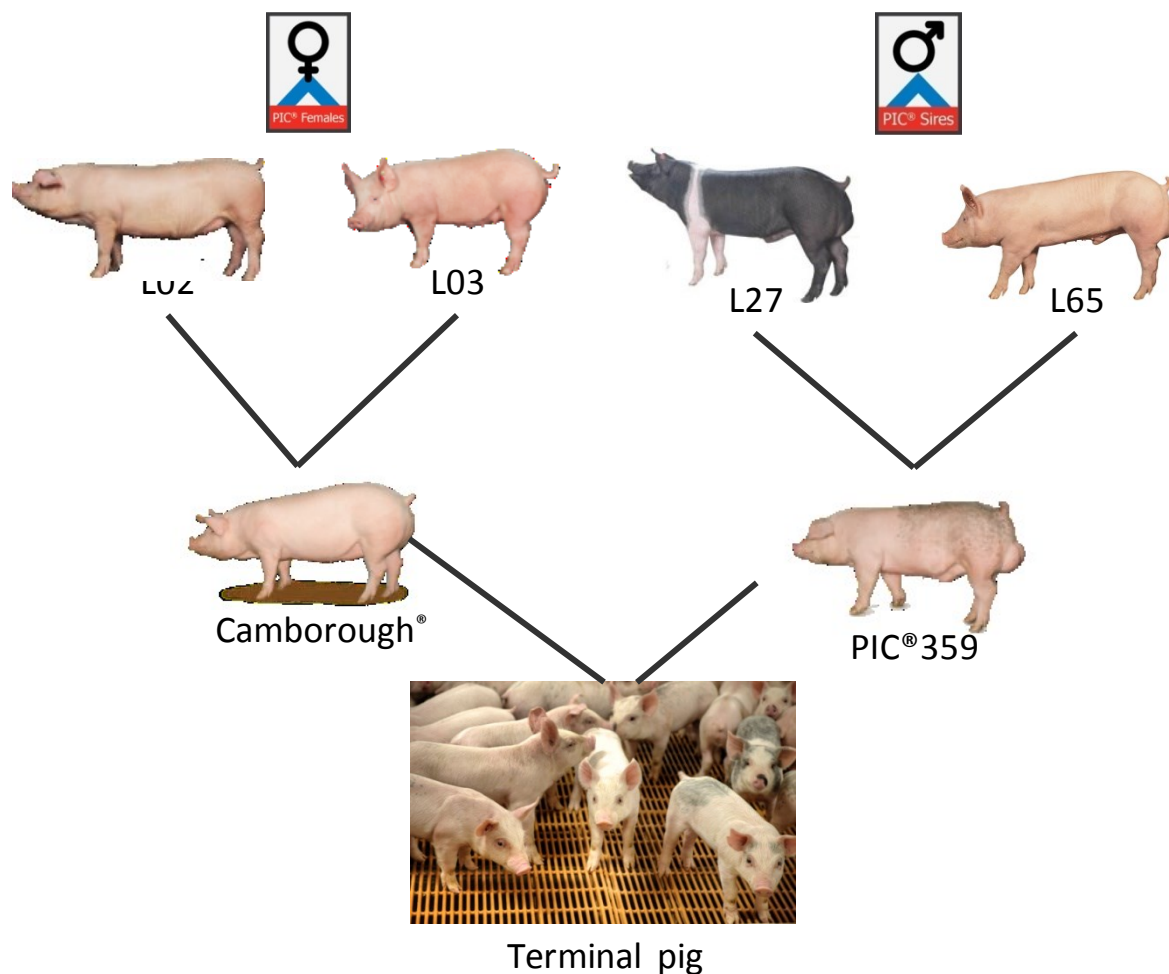
The promise of gene editing to improve both animal health and thereby farmers' output and income, as well as the sustainability of animal protein production are evident. These opportunities are closer to becoming a reality with the advent of facile gene editing technologies that allow precise and reproducible changes in animal genomes. These technologies have led to rapid progress in the science that supports these technological opportunities and renewed interest in the commercial application of these technologies in livestock. Success in the market place requires overcoming further challenges. Creation of, and adherence to, a predictable, science-based regulatory process; advancing these technologies with appreciation for the sensitivity of the public to animal well-being in agriculture; and simply meeting farmer expectations on elite livestock performance in a world of continuous genetic improvement are all challenges that must be overcome for this promise to be realised.

Figure 1. Relationship based genomic selection impact on rate of genetic improvement.



(1) An internal index to predict the marginal profit potential from PIC genetics for our customers

Figure 2. Example of line complexity in commercial pork production.



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